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Seroresponses to Virus-Like Particles of Human Papillomavirus Types 16, 18, 31, 33, and 45 in San People of Southern Africa

Dianne Marais,[1] Robert C. Rose,[2] Christopher Lane,[2] Sanet Aspinall,[3] Pieter Bos,[3] and Anna-Lise Williamson[1]*

[1]*Department of Medical Microbiology, UCT Medical School, Cape Town, South Africa*
[2]*Departments of Medicine and Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York*
[3]*Department of Virology, Medical University of South Africa, Medunsa, South Africa*

Virus-like particles (VLPs) of the high-risk human papillomavirus (HPV) types 16, 18, 31, 33, and 45 were used as antigen in enzyme-linked immunosorbent assay (ELISA) to determine the prevalence of serum IgG in a group of San people originally from Namibia, now residing in South Africa. The San children had low seroprevalence to all VLP types, but 26/115 (22.6%) of the children were seropositive to at least 1 VLP type. Among the adults, seroprevalence was significantly higher. The seroprevalence of antibodies in 101 San women to VLP-16 was 16.8%, VLP-18 18.8%, VLP-31 12.9%, VLP-33 17.8%, and VLP-45 22.8%. Five of the 11 men were seropositive: 2 for VLP-31, 1 for VLP-18, 1 for VLP-33, and 1 for VLP-45. Seroreactivity appeared to be type specific, except possibly to VLP-18 and -45. Of the adults, 50.5% were seropositive to at least 1 VLP type and 24.8% were seropositive to >1 VLP type. From this study, it is concluded that the San people are exposed to HPV-16, -18, -31, -33, and -45, with antibodies to VLP-45 being the most prevalent. ***J. Med. Virol. 60:331–336, 2000.***

KEY WORDS: serology; antibodies; HPV; VLP; San (bushmen)

INTRODUCTION

A number of different human papillomavirus (HPV) types have been established as oncogenic factors for cervical neoplasia [NIH Consens Statement, 1996], including HPV types 16, 18, 31, 33, 35, 39, 41, 42, 45, and 52 [Schiffman and Brinton, 1995]. It is estimated that 6% of the 9 million cases of cancer recorded worldwide each year could be attributed to HPV infection, with women in developing countries at particularly high risk of HPV-associated cancers [IARC/WHO, 1999]. Like South Africa, Namibia has one of the highest prevalence rates in the world for cervical HPV infection and cervical intraepithelial neoplasia (CIN) [Bloch et al., 1988]. However, at present there is little information regarding the most prevalent HPV types, or those associated with cervical disease, in the region of southern Africa. This information is a necessary requirement for the production of HPV vaccines, as a vaccine should protect against several types [IARC/WHO, 1999] and should reflect the prevalent HPV types in the area in which it will be utilized.

In 1989, at the end of the Angolan war, a group of San people, also known as bushmen, were translocated from Omega in northern Namibia and were settled at Schmidtsdrift, in the Northern Cape province of South Africa. This community now consists of about 4,500 people [Gaobepe et al., 1995]. These San people are the descendants of the original indigenous hunter–gatherer people of southern Africa [Deacon, 1992], whereas the Negroid population, who now dominate the region, only migrated there a few hundred years ago [du Toit et al., 1990].

Sera were available from the San people but no cervical specimens for DNA analysis, as is the situation for many African communities. Therefore, this study proposed to use HPV virus-like particle (VLP) serology to determine the exposure of the San people to 5 oncogenic HPV types. Seroreactivity to HPV VLPs is reported to be a better marker for cervical cancer risk than HPV DNA [Nonnenmacher et al., 1995, 1996], and populations with a high cervical cancer (CaCx) incidence have a higher HPV-16 antibody prevalence than those with a low CaCx incidence [Strickler et al., 1999].

Serologic assays based on HPV-16 VLPs (VLP-16)

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*Correspondence to: Dr. Anna-Lise Williamson, Department of Medical Microbiology, UCT Medical School, Observatory 7925, Cape Town, South Africa. E-mail: annalise@medmicro.uct.ac.za

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have been used successfully to detect present or past infection with HPV-16 [Kirnbauer et al., 1994; Wikström et al., 1995]. Antibodies to VLP-16 have also indicated persistent HPV infection [Wideroff et al., 1995] and predicted cervical disease [Dillner et al., 1995; Nonnenmacher et al., 1995; Marais et al., 1997]. The tests appear to be type specific, as those with HPV DNA are more likely to have antibodies to that specific type [Kirnbauer et al., 1994; Wideroff et al., 1995; Hamšiková et al., 1997; Tachezy et al., 1999]. Recently, a number of studies have shown that antibodies to VLPs from HPV-16, -18, and -33 were associated with an increased risk of CaCx [Wang et al., 1997]. Matsumoto et al. [1997] showed that antibodies to VLP-16, -18, and -58 are associated with CIN and CaCx. In other such studies, Dillner et al. [1996] indicated that antibodies to VLP-16, -18, and -33 were markers of sexual behavior, and Strickler et al. [1999] showed that VLP-16 antibodies indicate sexual HPV exposure in women.

The aim of this study was to obtain serologic evidence of exposure to 5 different high-risk HPV types (HPV-16, -18, -31, -33, and -45) in a community of San people originating from Namibia. Seroprevalence studies facilitate the documentation of exposure to the various HPV types prevalent in communities. These data would be helpful in providing the background information needed to design appropriate HPV vaccines for specific communities.

MATERIALS AND METHODS

Serum Samples

Sera of San volunteers from Schmidtsdrift were originally obtained in 1993 for a baseline epidemiologic study and controlled immunization program with 2 recombinant hepatitis B vaccines [Gaobepe et al., 1995]. At that time, families were informed of the purpose of the study and volunteers were included in the study after signed consent from the families. Of the original sera collected, only 244 specimens remained with sufficient sample volume for this study. These sera were derived from 115 children aged between 2 and 12 years, 17 teenagers, and 112 adults aged 20–83 years. The majority were female (61 children, 11 teenagers, and 101 adults). The average age of the children was 8 years (female children, average age 8.3 years; male children, average age 7.6 years). The average age of the 101 women was 38 years. Of the males, 54 were children, 6 were teenagers, and 11 were adults (male adults, average age 41.6 years).

Antigen

VLPs were produced in insect cells [Rose et al., 1993] from baculovirus-expressed recombinant L1 proteins of HPV-16, -18, -31, -33, and -45 and purified in CsCl gradients.

Enzyme-Linked Immunosorbent Assay (ELISA)

Sera were tested for antibodies at a 1:20 dilution by ELISA, using a VLP concentration of 10 μg/ml, as described previously [Marais et al., 1997]. Each serum was tested for reactivity to the 5 VLP types at the same time, on 2 HPV-VLP-coated wells for each VLP type, with an adjacent BPV-VLP well (total of 5 BPV-VLP-coated wells per serum). For each serum the optical density (OD) value obtained on the BPV-VLP-coated well was subtracted from the mean of 2 values obtained on the HPV-VLP-coated wells, giving a corrected OD value. This was done to eliminate background caused by antibodies to baculovirus, insect cell, and tissue culture proteins, which copurify with the VLPs. For each VLP type, the cutoff value was calculated from readings obtained from using the children's sera. The mean of the children's corrected OD values, plus 2 standard deviations (mean + 2 SD), after the elimination of outliers as previously described [Marais et al., 1997], constituted the final cutoff value.

Data Analysis

Data were statistically analyzed by χ^2 test using Epi Info Version 5 (Centres for Disease Control, Epidemiology Program Office, Atlanta, GA). The significance level for assessing deviations from the tested hypothesis was $P = 0.05$ for all tests. Scatterplot analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA).

RESULTS

In ELISA the San peoples' sera indicated a high level of background reactivity to VLPs, much higher than that of Cape Town blood donors and children [Marais et al., 1997]. It was not known whether this was due to antibodies to insect proteins in the San sera or to the storage of the serum, but emphasized the importance of the consideration of different cut points for different population groups. ELISA cutoff values were determined using San children's sera, as it has been shown that young children have low levels of antibodies to VLPs [Marais et al., 1997; Hamšiková et al., 1998; af Geijersstam et al., 1999]. The mean OD value obtained for the reactivity to the BPV-VLP was 0.648 (SD 0.32). Boxplot analysis of the uncorrected OD values obtained for the San sera in the VLP ELISAs is shown in Figure 1. The prevalence of serum antibodies to each of the 5 VLP types among the San people is shown in Table 1. The seroprevalence among the children was low (<8%) for all the VLP types, but 2–3 times higher among the San adults.

Of the 26 children who were seropositive, 9 were positive to >1 VLP type and all but 2 of the 9 multiple seropositivities (Table II) were in the male group. A higher percentage of the children <8 years of age were seropositive (28%) than those >7 years of age (18%; $P = 0.05$) (Table II). A significantly higher percentage of the male children (33%) were seropositive compared with the female children (13%; $P = 0.0007$).

The seroprevalence of the San women to single and multiple VLPs is presented in Table III. Of the seropositive women, almost half had antibodies to a single VLP type, whereas the rest were seropositive to mul-

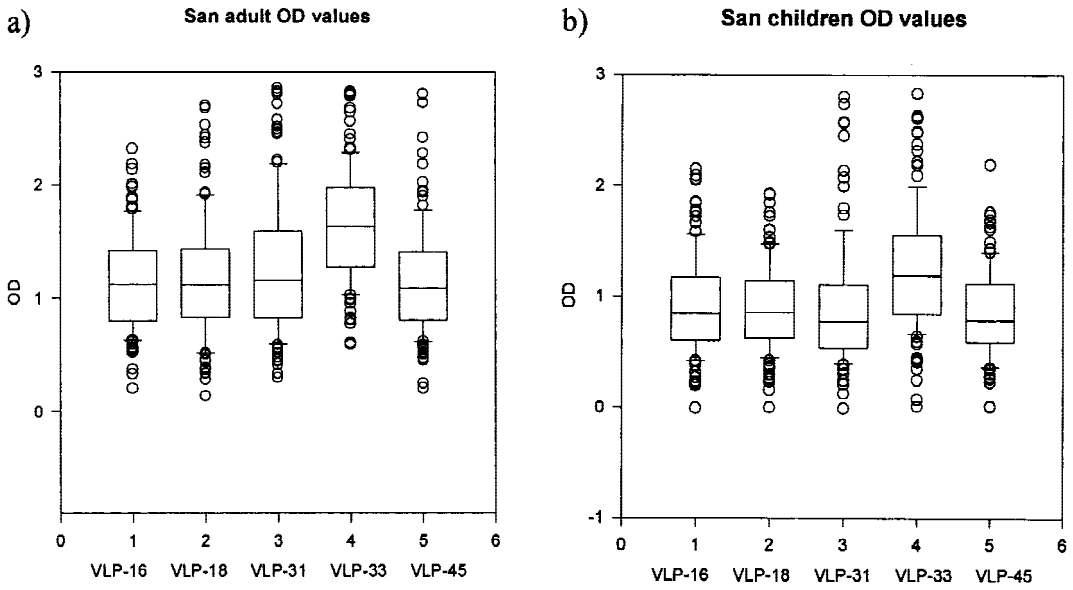

Fig. 1. Boxplot analysis of the uncorrected OD values obtained in VLP-16, -18, -31, -33, and -45 ELISAs for **(a)** San adults and **(b)** San children. ELISA cutoff values as determined using the San children's sera were 0.73 for VLP-16, 0.80 for VLP-18, 1.3 for VLP-31, 1.4 for VLP-33, and 0.7 for VLP-45.

TABLE I. Seroprevalence of IgG Antibodies to VLP-16, VLP-18, VLP-31, VLP-33, and VLP-45 Among the San People

	No. seropositive (%)		
VLP type	Children (N = 115)	Teenagers (N = 17)	Adults (N = 112)
VLP-16	7 (6.1)	2 (11.8)	18 (16.1)
VLP-18	8 (7.0)	2 (11.8)	19 (17.0)
VLP-31	7 (6.1)	1 (5.9)	15 (13.4)
VLP-33	9 (7.8)	0	20 (17.9)
VLP-45	7 (6.1)	0	24 (21.4)

tiple VLPs. Seropositive women were assessed in 2 age groups, either <36 years or >35 years of age, with regard to reactivity to each of the 5 VLP types or combinations of VLP types (Table III). A significantly greater number of older women (31/50, 62%) were positive compared with the younger group (20/51, 39%) ($P = 0.02$). Five of the 11 men were seropositive: 2 were positive for HPV-31, 1 was positive for HPV-18, 1 was positive for HPV-33, and 1 was positive for HPV-45 VLP antibodies.

Because so many individuals showed seropositivity to a number of VLP types, the occurrence of seropositivity to increasing numbers of VLP types was assessed in San children, teenagers, and adults (Fig. 2). The majority of the children (77.4%) were seronegative, but of those that did show seroreactivity, most reacted with only 1 of the VLP types. In contrast, the presence of antibodies to 2, 3, or 4 different VLP types was more evident in the adults (22.7%). However, more than half of the women who were seropositive had antibodies to only 1 VLP type (26/51, 50.9%), indicating a type-specific response and supporting the probability that these women were infected with multiple types rather than displaying cross-reacting antibodies.

In an attempt to ascertain possible serologic cross-reactivity between the different VLP types, scatterplot analysis of the corrected OD values for each VLP type was compared with the other VLP types (Fig. 3). A weak correlation was found between VLP-18 and VLP-45 in the San adult group ($R^2 = 0.466$). For all of the other VLP types, the R^2 value was <0.1 when compared with one another.

DISCUSSION

The first trials of candidate HPV VLP vaccines in humans are under way [IARC/WHO, 1999]. This raises the hope of the availability of HPV vaccines in the near future. Indications are that the immunity induced to HPV is type specific [Rose et al., 1994; Roden et al., 1996] and vaccination with 1 type may be unlikely to protect against infection with another type. A study of the HPV types in the southern African population is necessary to determine exposure to the major oncogenic types that exist, if HPV vaccines are to be introduced. This study examined the differences in seropositivity to 5 oncogenic HPV VLP types among the San people.

San children had low seropositivity to the individual VLPs and this is comparable with results described for HPV-16 [Marais et al., 1997]. However, if the results of the tests to individual VLPs were combined, then 22.6% of the San children were seropositive to at least 1 HPV type. Similarly, the seroreactivity of 115 children in Cape Town to at least 1 type was 27.3% (unpublished data). This is a relatively large number of children who have been exposed to high-risk HPV types. Transmission of HPV from mother to infant has been described [Cason et al., 1995; Puranen et al., 1997] and may account for some of these observations.

TABLE II. Seropositivity in San Children to a Single VLP Type or Combinations of VLP Types According to Age and Gender

VLP types	No. of seropositivities			
	Male (n = 54)	Female (n = 61)	Age <8 years (n = 46)	Age >7 years (n = 69)
16	3	2	4	1
18	1	1	0	2
31	1	2	0	3
33	5	1	3	3
45	1	0	1	0
16, 18	1	0	1	0
16, 45	1	0	0	1
18, 31, 33, 45	1	0	0	1
18, 31, 45	1	0	0	1
18, 45	2	1	2	1
31, 33	1	1	2	0
Total	18/54 (33%)	8/61 (13%)	13/46 (28%)	13/69 (18%)

TABLE III. Seropositivity in San Women to a Single VLP Type or Combinations of VLP Types According to Age

VLP types	No. of seropositivities		
	Age <36 years (n = 51)	Age >35 years (n = 50)	Total (n = 101)
16	2	3	5
18	2	3	5
31	3	1	4
33	2	5	7
45	1	4	5
16, 18, 31, 45	1	0	1
16, 18, 33, 45	1	1	2
16, 18, 45	1	2	3
16, 31	2	1	3
16, 31, 33	0	1	1
16, 33	0	1	1
18, 31, 33, 45	1	0	1
18, 33, 45	0	1	1
18, 45	2	4	6
33, 45	2	1	3
31, 33	0	2	2
31, 45	0	1	1
Total	20/51 (39%)	31/50 (62%)	51/101 (50.5%)

More of the children <8 years of age were seropositive (13/46, 28.2%) than those >7 years of age (13/69, 18.8%), suggesting that seropositivity drops off with age and that infection occurs early in life. The reason for the high seropositivity in male children (18/54) compared with female children (8/61) is not clear. Although a gender-related mode of transmission may be implicated in the children, fewer adult males have antibodies to VLPs than do adult women [Strickler et al., 1999].

Almost half the women and more than a third of the children who were HPV VLP seroreactive were positive to >1 VLP type. The relevance of antibodies to >1 type of VLP can be explained in terms of multiple infections or serologic cross-reactivity. Individuals could accumulate multiple HPV infections over time, or the immune response could change with time. It has been postulated that the number of epitopes detected could increase with repeated antigenic stimulation, from 1 immunodominant, type-specific epitope to include some

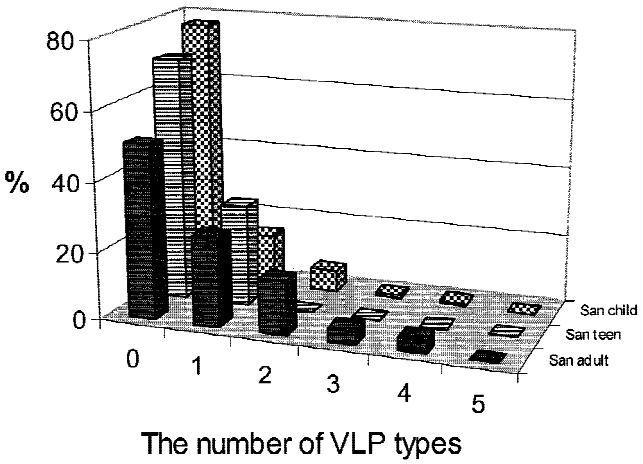

Fig. 2. The percentage of seronegative individuals in each group and the percentage of those seropositive to 1–5 VLP types.

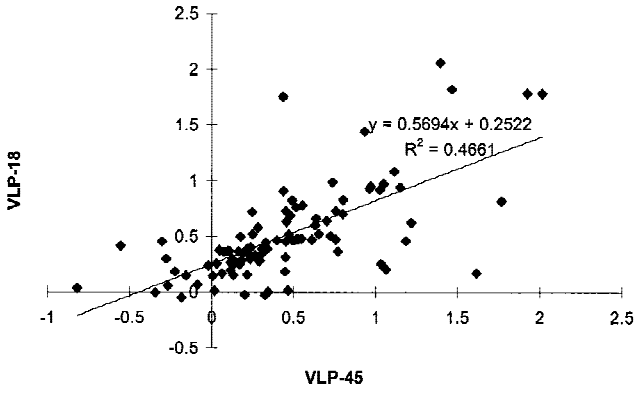

Fig. 3. A comparison by scatterplot analysis of the seroreactivity of San women to VLP-18 and VLP-45. The trend-line bisection of the y-axis could be an indication of higher background reactivity to the one VLP preparation with relation to the other.

cross-reactive epitopes [Wang et al., 1997]. This may explain the simultaneous detection of antibodies to VLP-18 and VLP-45, where seroreactivity to 1 type indicates a type-specific immunodominant epitope. However, there was also a significant concurrent seroreac-

tivity to both VLP-18 and VLP-45 in 14/25 women. This combination of seroreactivity to VLP-18 and -45, correlates with their phylogenetic relatedness and could indicate cross-reactivity.

Previous studies [Bloch et al., 1988] on the prevalence of genital tract HPV in the Namibian region showed a very high incidence of both CIN and genital HPV types in the people of that area. The San group in our investigation included people from the same regions of Namibia as in that study. The San women had seroprevalence levels for VLP-18, -33, and -45 which were higher than those reported for blood donors in Cape Town (VLP-18 10.5%, VLP-33 8.4%, and VLP-45 13.7%) (unpublished data), indicating a higher exposure to these HPV types in the San community compared with the Cape Town blood donors. High seroprevalence of antibodies to VLP-18 and VLP-33 among normal healthy control women has been described [Chua et al., 1996] which did not confer an increased risk of cervical disease, whereas seroprevalence to VLP-16 conferred a 3-fold increased risk of developing CIN. In a study comparing VLP-16 seroprevalence in blood donors in the United States and Jamaica [Strickler et al., 1999], where there is a 3-fold higher incidence of CaCx, age-adjusted seroprevalence rates for VLP-16 were 12% and 24%, respectively. Blood donors in Cape Town have a VLP-16 seroprevalence of 25.5% (unpublished data). The San VLP-16 seroprevalence was 16.8%, suggesting a lower exposure to HPV-16 and consequently a lower risk of CaCx, which correlates with the reported cytologic findings of the San women. In general, the San people have only 1 sexual partner, once married; although the younger San people may have >1 sexual relationship, a high degree of promiscuity is not common. Only 1 case of CaCx has been reported in the last 5 years in the Schmidtsdrift community of 4,500 people (Captain Pretorius, personal communication). However, as seropositivity to oncogenic HPV types was evident in 50% of the San women, cervical infection in the women or infection at sites other than the cervix cannot be ruled out.

A limited amount of data is available on the HPV types present in South African communities. In Cape Town, HPV-16 has been reported to be the predominant type in women with CIN and CaCx [Williamson et al., 1989, 1994], while in women with normal cytology, the predominant type is HPV-18 [Ramesar et al., 1996]. In situ hybridization studies to establish which HPV types were associated with CIN and CaCx in Durban indicated that HPV-16 and HPV-18 were the predominant types but the minor types of HPV were also present in this region [Cooper et al., 1991a, b]. Some information is available on HPV types present in central and northern African countries. Bosch et al. [1995] indicated a significant increase in HPV-45 DNA among CaCx patients in African countries in their study, compared with other areas of the world. HPV-18 DNA in Tanzania [ter Meulen et al., 1992] and Uganda [Schmauz et al., 1989] has been found to be more prevalent among CaCx and noncancer patients than other areas of the world. In Senegal, a high proportion of patients with cervical lesions was shown to be infected with HPV-18 (39%) and HPV-45 (10%), and HPV-18 DNA was found in 7% of pregnant women [Chabaud et al., 1996].

This study has contributed information on the presence of antibodies to 5 HPV VLP types in San people indicating that all 5 HPV types are present in this population. These findings regarding the San people corroborate other evidence that HPV-18 and HPV-45 have a high prevalence in certain areas of Africa. It remains to be established whether San women with antibodies to these VLP types are at increased risk of cervical disease.

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